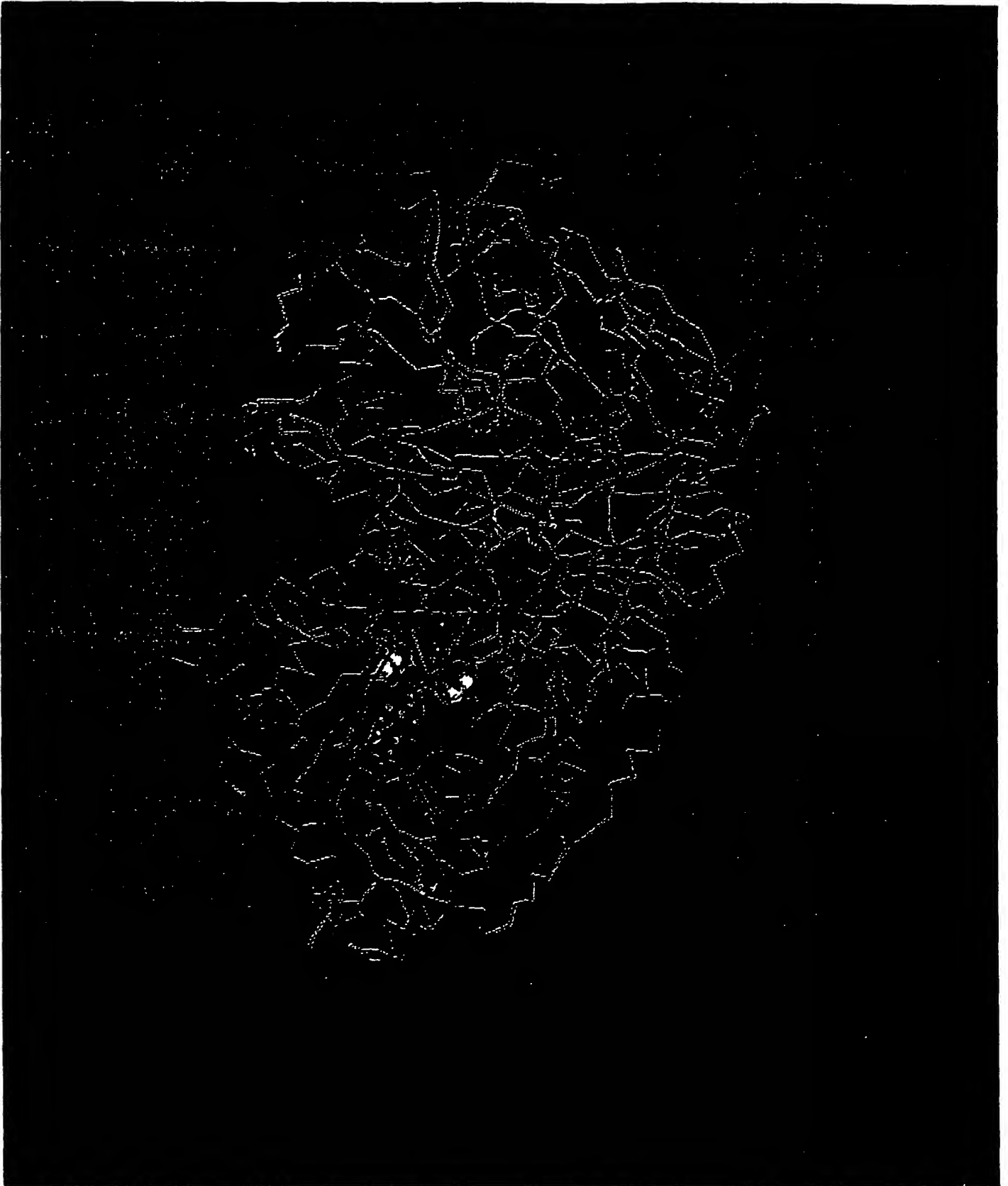
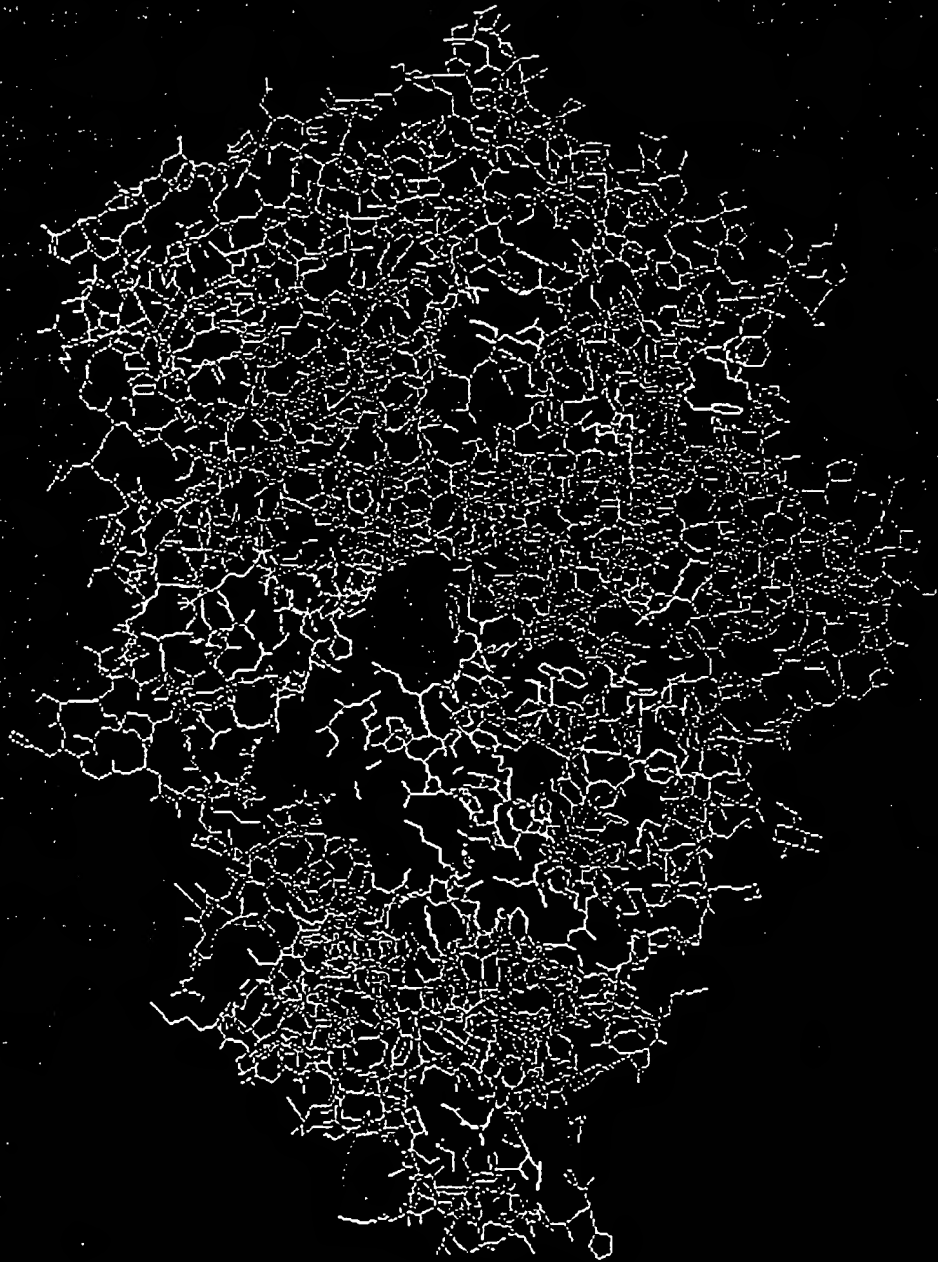


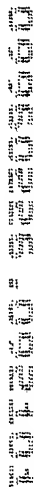
FIG. 20



12243.19USU1







## pMT/BIP-N-HIS-dGMII [851 to 4042] -phase Translation

DNA sequence 6642 b.p. TCGCGG...CG ... AGGCCCTTTCGT circular

N-HIS added to pMTV5HisA (b/t BglII/EcoRI) for drosophila expression of tagged proteins.  
Add drosophila dGMII from clone D11 (shortened 3') DAK/Tara 15-3-99

Inventor: ROSE et al.  
Docket No.: 12243.19USUI  
Title: MANNOSIDASE STRUCTURES  
Attorney Name: Douglas P. Mueller (Reg. No. 30,300)  
Phone No.: 612.371.5237  
Sheet 6 of 20

851/1 atg AAG TTA TGC ATA TTA CTG GCC GTC GTG 881/11 GCC TTT GTT GGC CTC TCG CTC GGG aga tct 911/21 agc cac cat cat cat cat cac gga gAA TTC  
M K L C I L L A V V A F V G L S L G R S S H H H H H H G E F  
941/31 gac gat cca ata aga cct cca ctt aaa gtg 971/41 gct cgt tcc ccg agy cca ggg caa tgc caa 1001/51 gat gtg gtc caa gac gtg ccc aat gtg gat  
D D P I R P P L K V A R S P R P G Q C Q D V V Q D V P N V D  
1031/61 gta cag atg ctg gag cta tac gat cgc atg 1061/71 tcc ttc aag gac ata gat gga ggc gtg tgg 1091/81 aaa cag ggc tgg aac att aag tac gat cca  
V Q M L E L Y D R M S F K D I D G G V W K Q G W N I K Y D P  
1121/91 ctg aag tac aac gcc cat cac aaa cta aaa 1151/101 gct ttc aag gac ata gat gga ggc gtg tgg 1181/111 cct gga tgg att cag acg ttt gag gaa tac  
L K Y N A H H K L K V F V V P H S H N D P G W I Q T F E E Y  
1211/121 tac cag cac gac acc aag cac atc ctg tcc 1241/131 aat gca cta cgg cat ctg cac gac aat ccc 1271/141 gag atg aag ttc atc tgg gcg gaa atc tcc  
Y Q H D T K H I L S N A L R H L H D N P E M K F I W A E I S  
1301/151 tac ttt gct cgg ttc tat cac gat ttg gga 1331/161 gag aac aaa aag ctg cag atg aag tcc att 1361/171 gta aag aat gga cag ttg gaa ttt gtg act  
Y F A R F Y H D L G E N K K L Q M K S I V K N G Q L E F V T  
1391/181 gga gga tgg gta atg ccg gac gag gcc aac 1421/191 tcc cac tgg cga aac gta ctg ctg cag ctg 1451/201 acc gaa ggg caa aca tgg ttg aag caa ttc  
G G W V M P D E A N S H W R N V L L Q L T E G Q T W L K Q F  
1481/211 atg aat gtc aca ccc act gct tcc tgg gcc 1511/221 atc gat ccc ttc gga cac agt ccc act atg 1541/231 ccg tac att ttg cag aag agt ggt ttc aag  
M N V T P T A S W A I D P F G H S P T M P Y I L Q K S G F K  
1571/241 aat atg ctt atc caa agy acg cac tat tgg 1601/251 gtt aag aag gaa ctg gcc caa cag cga cag ctt gag ttc ctg tgg cgc cag atc tgg gac  
N M L I Q R T H Y S V K K E L A Q Q R Q L E F L W R Q I W D  
1661/271 aac gga ggg gac aca gct ctc ttc acc cac 1691/281 atg atg ccc ttc tac tgg tac gac att cct cat acc tgt ggt cca gat ccc aag gtt tgc  
N K G D T A L F T H M M P F Y S Y D I P H T C G P D P K V C  
1751/301 tgt cag ttc gat ttc aaa cga atg ggc tcc 1781/311 ttc ggt ttg agt tgt cca tgg aag gtg ccg ccg cgt aca atc agt gat caa aat gtg gca  
C Q F D F K R M G S F G L S C P W K V P P R T I S D Q N V A  
1841/331 gca cgc tca gat ctg ctg gtt gat cag tgg 1871/341 aag aag aag gcc gag ctg tat cgc aca aac gtg ctg ctg att ccg ttg ggt gac gac ttc  
A R S D L L V D Q W K K K A E L Y R T N V L L I P L G D D F  
1931/361 cgc ttc aag cag aac acc gag tgg gat gtg 1961/371 cag cgc gtg aac tac gaa agy ctg ttc gaa cac atc aac agc cag gcc cac ttc aat gtc  
R F K Q N T E W D V Q R V N Y E R L F E H I N S Q A H F N V  
2021/391 cag gcy cag ttc ggc aca ctg cag gaa tac 2051/401 ttt gat gca gtg cac cag gcg gaa agy gcg gga caa gcc gag ttt ccc acg cta agc ggt  
Q A Q F G T L Q E Y F D A V H Q A E R A G Q A E F P T L S G  
2111/421 gac ttt ttc aca tac gcc gat cga tgg gat 2141/431 aac tat tgg agt ggc tac tac aca tcc cgc ccg tat cat aag cgc atg gac cgc gtc ctg  
D F F T Y A D R S D N Y W S G Y Y T S R P Y H K R M D R V L  
2201/451 atg cac tat gta cgt gca gca gaa atg ctt 2231/461 tcc gcc tgg cac tcc tgg gac ggt atg gcc cgc atc gag gaa cgt ctg gag cag gcc cgc  
M H Y V R A A E M L S A W H S W D G M A R I E E R L E Q A R  
2291/481 agg gag ctg tca ttg ttc cag cac cac gac 2321/491 ggt ata act ggc aca gca aaa acg cac gta gtc gtc gac tac gag caa cgc atg cag gaa  
R E L S L F Q H H D G I T G T A K T H V V V D Y E Q R M Q E  
2381/511 gct tta aaa gcc tgt caa atg gta atg caa 2411/521 cag tgg gtc tac cga ttg ctg aca aag ccc tcc atc tac agt ccg gac ttc agt ttc tgg  
A L K A C Q M V M Q Q S V Y R L L T K P S I Y S P D F S F S  
2471/541 tac ttt acg ctc gac gac tcc cgc tgg cca 2501/551 gga tct ggt gtg gag gac agt cga acc acc ata ata ctg ggc gag gat ata ctg ccc tcc  
Y F T L D D S R W P G S G V E D S R T T I I L G E D I L P S  
2561/571 aag cat gtg gtg atg cac aac acc ctg ccc 2591/581 cac tgg cgg gag cag ctg gtg gac ttt tat gta tcc agt ccg ttt gta agc gtt acc gac  
K H V V M H N T L P H W R E Q L V D F Y V S S P F V S V T D  
2651/601 ttg gca aac aat ccg gtg gag gct cag gtg 2681/611 tcc ccg gtg tgg agc tgg cac cac gac aca ctc aca aag act atc cac cca caa gcc tcc  
L A N N P V E A Q V S P V W S W H H D T L T K T I H P Q G S  
2741/631 acc acc aag tac cgc atc atc ttc aag gct 2771/641 cgg gtg ccg ccc atg ggc ttg gcc acc tac gtt tta acc atc tcc gat tcc aag cga gag  
T T K Y R I I F K A R V P P M G L A T Y V L T I S D S K P E  
2831/661 cac acc tgg tat gca tgg aat ctc ttg ctc 2861/671 cgt aaa aac ccg atc tgg tta cca ttg ggc caa tat ccg gag gat gtg aag ttt ggc gat  
H T S Y A S N L L L R K N P T S L P L G Q Y P E D V K F G D  
2921/691 cct cga gag atc tca ttg cgg gtt ggt aac 2951/701 gga ccc acc ttg gcc ttt tgg gag cag ggt ctc ctt aag tcc att cag ctt act cag gat  
P R E I S L R V G N G P T L A F S E Q G L L K S I Q L T Q D  
3011/721 agc cca cat gta ccg gtg cac ttc aag ttc 3041/731 ctc aag tat ggc gtt cga tgg cat ggc gat aga tcc ggt gcc tat ctg ttc ctg ccc aat  
S P H V P V H F K F L K Y G V R S H G D R S G A Y L F L P N  
3101/751 gga cca gct tgg cca gtc gag ctt ggc cag 3131/761 cca gtg gtc ctg gtg act aag ggc aaa ctg gag tgg tcc gtg agc gtg gga ctt ccg agc  
G P A S P V E L G Q P V V L V T K G K L E S S V S V G L P S  
3191/781 gtg gtg cac cag acg ata atg cgc ggt ggt 3221/791 gca cct gag att cgc aat ctg gtg gat ata ggc tca ctg gac aac acg gag atc gtg atg  
V V H Q T I M R G G A P E I R N L V D I G S L D N T E I V M  
3281/811 cgc ttg gag acg cat atc gac agc ggc GAT 3311/821 ATC ttc tac acg gat ctc aat gga ttg caa ttt atc aag agy ccg cgt ttg gac aaa tta  
R L E T H I D S G D I F Y T D L N G L Q F I K R R R L D K L



Figure 7

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ALIGNMENT OF EXPRESSED SECRETED DROSPHILA MANNOSIDASE  
WITH HUMAN MANNOSIDASE  
Percent Similarity: 52.157 Percent Identity: 43.039

1 .....RSSHHHHGEFD 13  
51 LQEKIDHLERLLAENNEIISNIRDSVINLSEVEDGPKSSQSFQAGS 100  
14 DPIRPLKVARSPRCQCDWVD.VPNVDVQMLELYDRMSFDIDGGV 62  
101 HL..PSQLSUSVDTADCLFASGSGSNSDVQMLDVYLSIFNPDGCV 148  
63 KQGNIKYDPLKYNAAHKLKVFVPHSHNDPGWIOTFEYYOHDTHKILS 112  
149 KQGFDTIYESNEDT.EPLQVVFVPHSHNDPGWLTATFNDYFRDKTQYIFN 197  
113 NALRHLNDPENKFIWAEISYFARFYHDLGENKKLQMKSIKNGQLEFVT 162  
198 NMVLKLEDSSRKFIWSEISYLSKWDIIDIQKDAVKSLEIENGQLEIVT 247  
163 GGWMPDEANSHWRNVLLQLTEGOTLWKQFMNVPTASWAIDPFHSPFM 212  
248 GGWMPDEATPHYFALIDQLIEGHQWLENNIGVKPRSGWAIDPFHSPFM 297  
213 PYILQSGFKNNMLIQRTYHSVKKELAQORLEFLMRQIWDNKGDTALFTH 262  
298 AYLLNRAGLSHMLIQRVHVAKKHFAHKLTEFFWRQWDLGVSVDILCH 347  
263 MPPFYSDIPHTCGPDKVCQCFDKRMGSGFLSCFPKVPPTISDQNV 312  
348 MPPFYSDIPHTCGPDKVCQCFDKRLPGGRFGCFGWVPPETIHGPNVQ 397  
313 ARSLLVQWKKKAEYRTNVLPLGDDFRKQNTWQVRVNYERLFE 362  
398 SRARMLLDQYRKSLFRTKVLAPLGDDFRYCEVTEWDLQFNKYQQLFD 447  
363 HINQAHFVQAFGLQETFDVHQAERA....QAEFPTLSGDFFTYA 408  
448 YMSQSKFKVKIQFTLSDFDALKADQTDQDKGQSMFVLSGDFFTYA 497  
409 DRSDNYWGYTSRPHKRDVLMHYVRAAEHLASW....HSWD.... 449  
498 DRDDHYWGYTSRPHKRDVLMHYVRAAEHLASW....HSWD.... 449  
450 GMARIEERLEQARRELSTFQHDGIGTAKTHVVVDYEQRMQEAALKACQ 499  
548 LSSSLYTALTEARNLGLFQHDGIGTAKTHVVVDYEQRMQEAALKACQ 499  
500 VMQSVYRLTKPSIYSPDFSFYFTLDDSRWPGSGVEDSRTIILGEDI 549  
598 IIGNSAFLIGDKLTYSPTDFLENDLKQKQSDSLFQKNIIRLSAE. 646  
550 LPSKVAAMHNTLPHWFEQVDFVYSSPFVSTDLANNPVEAQVSPVMSWH 599  
647 ..PRYLVVYVNPLEQDRISLSVSVSSPTVQVFSAGKPVVEVQVSAVW... 691  
600 HDTLTKTHPQSTTKYRIIFKARVPNGLATYVLTISDSKPEHTSYASN 649  
692 .DT.ANTI....SETAYEISFRAHIPPLGLKVYKILESSASS...NSHLAD 732  
650 LLLRNKPTSLPLQYEPEDVKFGDPREISLRVCNGPTLAFSEQGLKSIQL 699  
733 YVLYKNKVE.DSGIFTIKNNINTEEGITLE.NSFVLLRFDDQTGLMKQMT 780  
700 TQDSPHVPVHFVKFLKGVSHGDRSGAYLFLPNGPASP.VELGQPVVLVT 748  
781 KEDGKHVEYVQVSWYGTGTTIKRDKSGAYLFLPDGNAKPYVYTPPFVRVT 830

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749 KGKLESVSGLPSPVWHQIM.....RGGAPEIRNLVDIGSLDNTFIM 792  
831 HGRIYSEVTCFFDHVTHRVLYHIQIEGQSVESNIVDIRKVVNRIAM 880  
793 RLETHIDSGDIFYTDLNGLQFIKRRRLDKLPQANYPIPSGMFIEDANT 842  
881 KISSDIKSNRFFYTDNGYQIQPRMTLSKLPQANVPMTTWAYIQDAKH 930  
843 RLTLTQPLQGGSSLASGELEIMQDRRLASDDERGLGOGVLDNKPKVLHIY 892  
931 RLTLTSAQSLGVSLSNSGQIEVIMDRMLQDDNRGLEQIQDNKITANLF 980  
893 RLYLEKNNVCVRPSKLHPAGYLTSAAHKASQSLDPL....DKFIFAENE 938  
981 RILLEKRSVNTTEEEKSVSPSLLSHITSSLMNHVPVPMANKF....SSP 1027  
939 WIGAQQGFGDHPASAREDLDSVSMRLTK..SSAKTORVGYVVLHRTNLMQ 986  
1028 TLELQGEFSPLOQSSLPDCDIHLVNLRTIQSKVGNHGSNEAALILHRKG.FD 1076  
987 C.....GTPE..EHTQ.KLDVCHLLPN..VACERTTTLTFLQNLHLHLDGM 1026  
1077 CRFSSKGTGLFCSTTQCKILVQKLLANKFIVESLTPSSLSLMHSPPGTQNI 1126  
1027 VAPEVCMPMETAAAYVSSHSS 1045  
1127 SEINLSPMEISTFRIQLR. 1144



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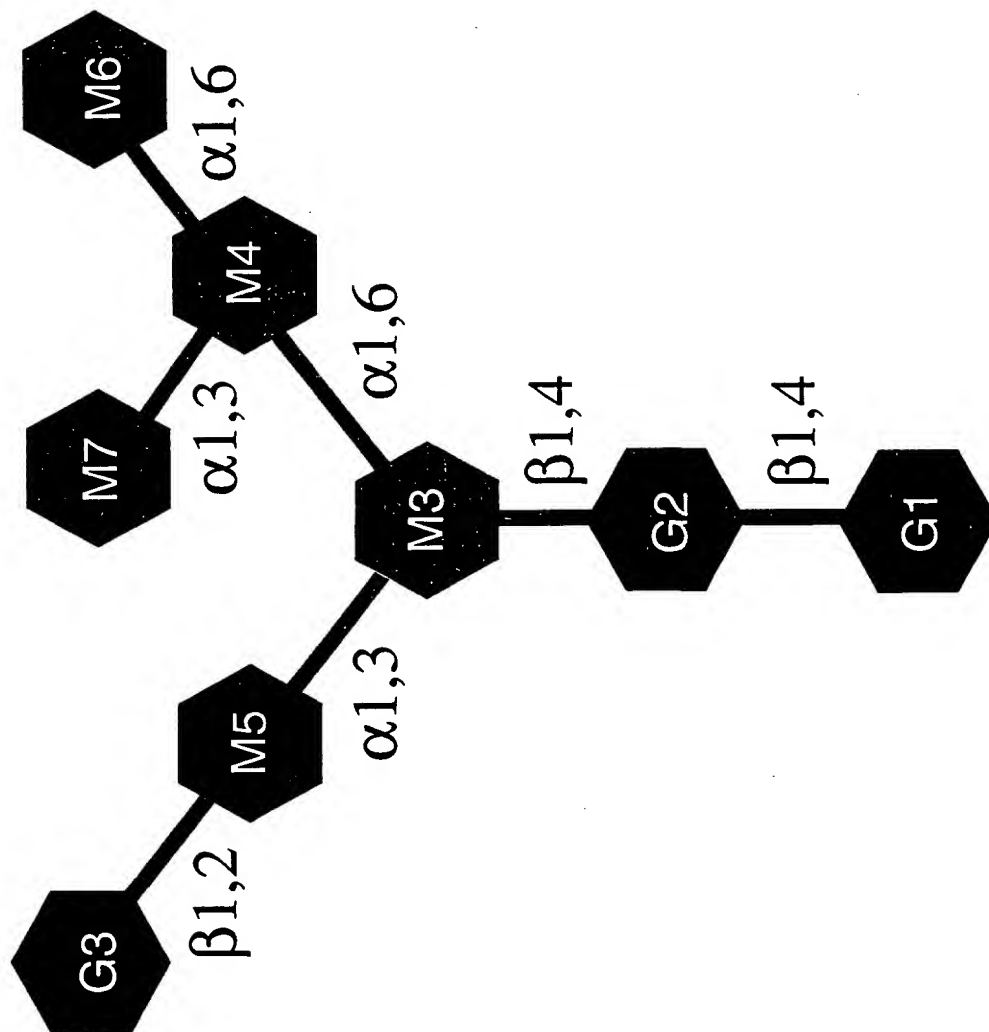


Figure 8B

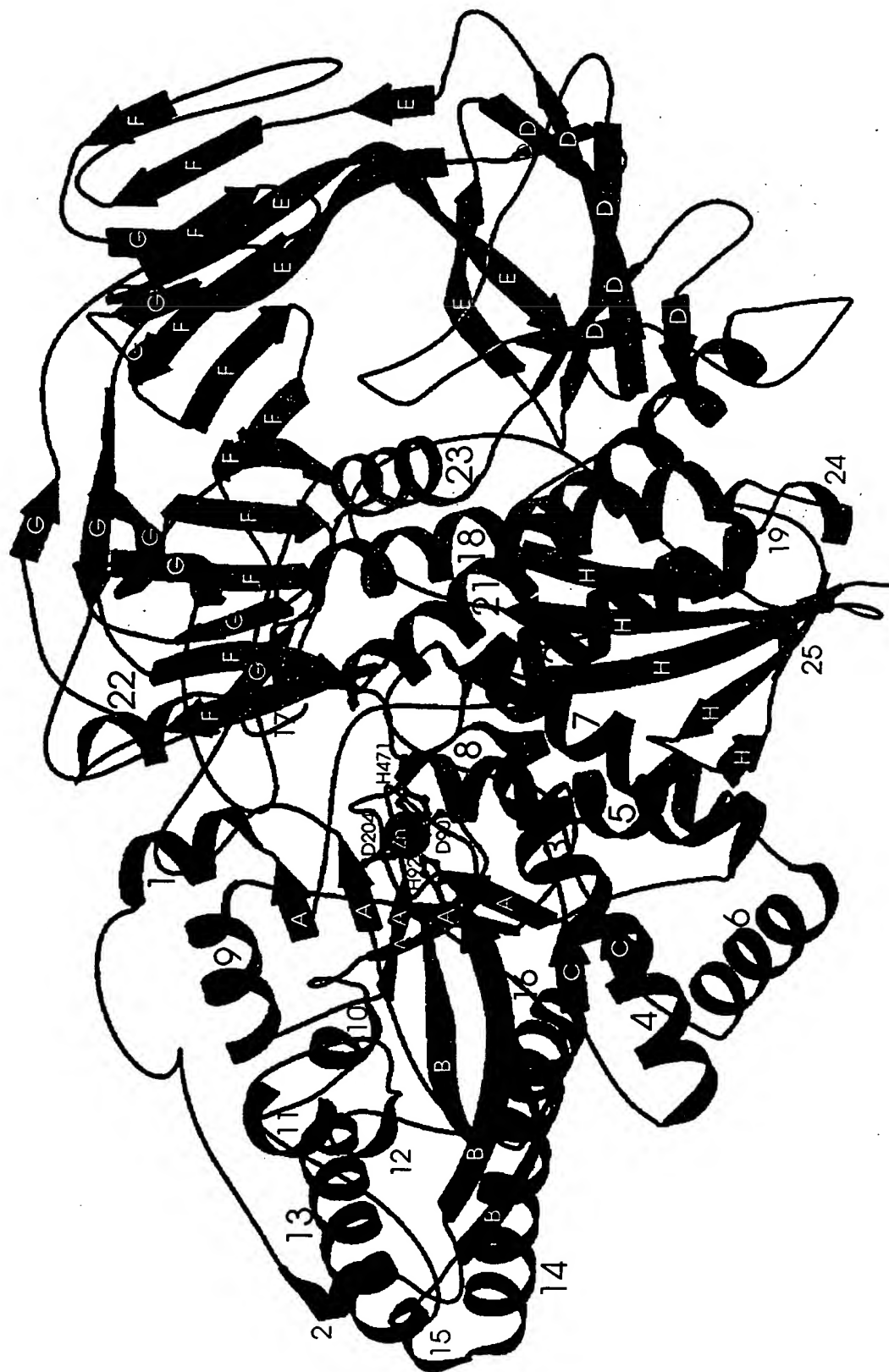




Figure 8C

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Fig. 9A



Fig. 9B

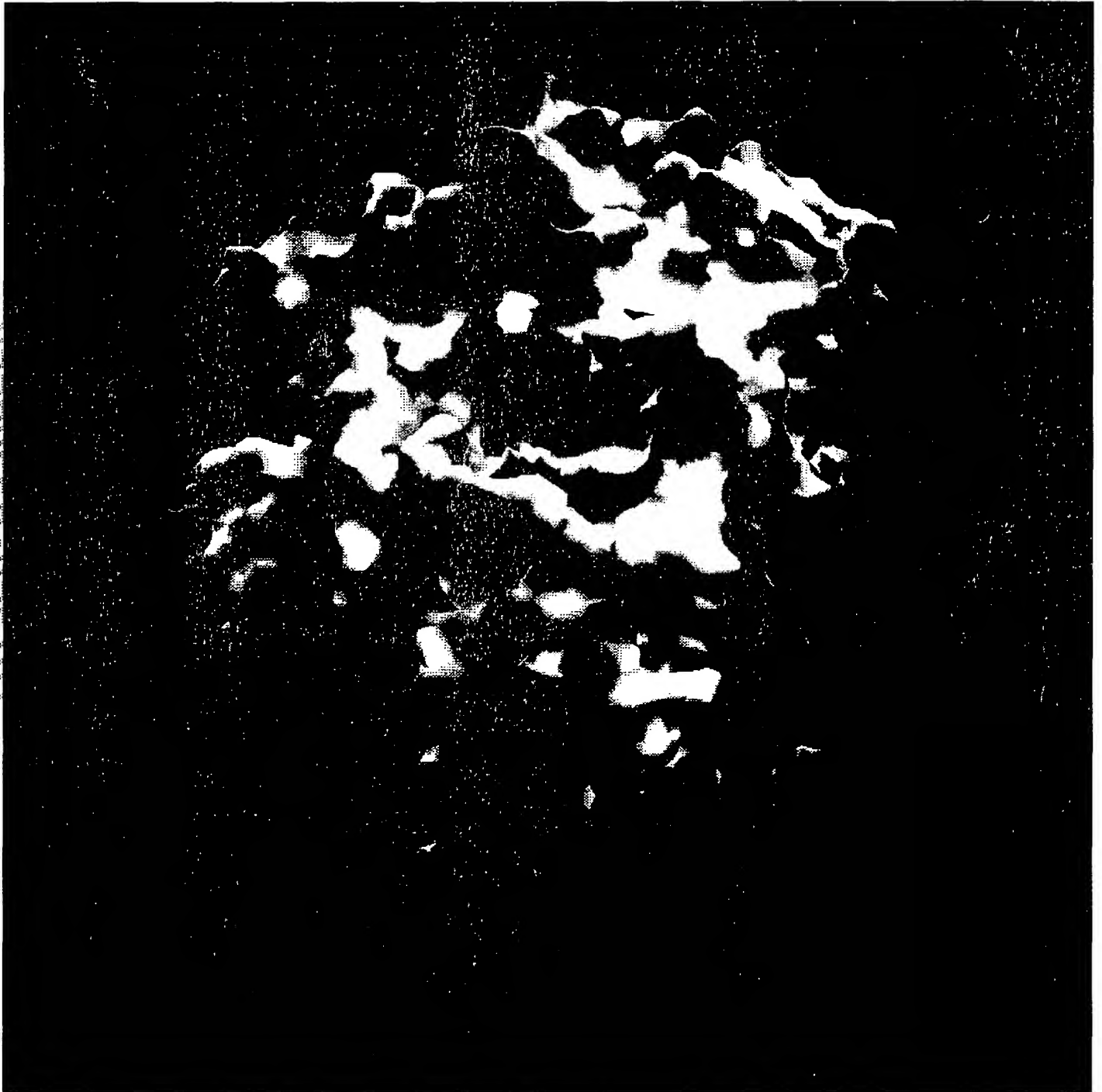
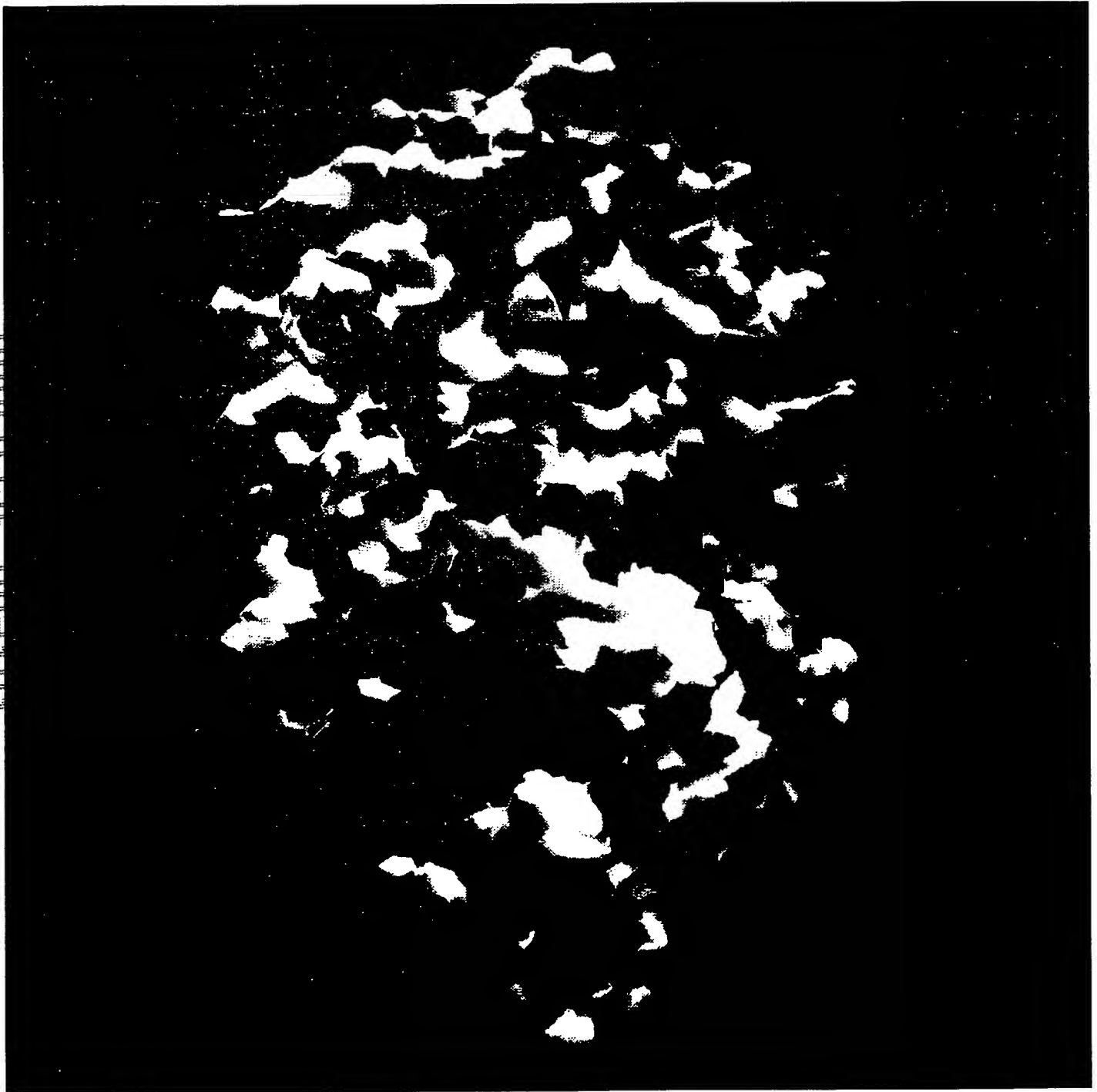


Fig. 9C



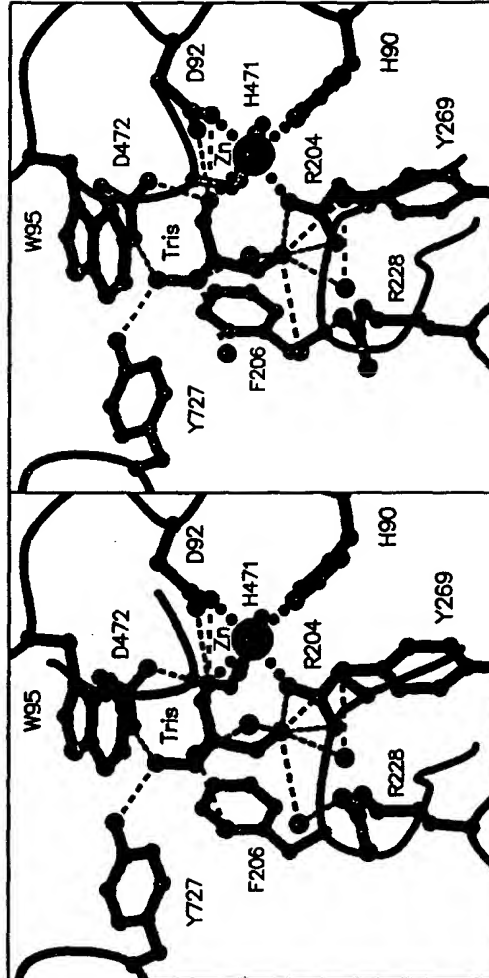


Figure 10A

FIG. 10A: MOLECULAR MODEL OF A ZINC-BINDING SITE IN A PROTEIN. THE ZINC ATOM IS COORDINATED BY RESIDUES D92, H471, R204, AND F206. OTHER RESIDUES SHOWN INCLUDE W95, D472, Y727, TRIS, R228, H90, AND Y269. DASHED LINES INDICATE COORDINATION BONDS AND HYDROGEN BONDS.

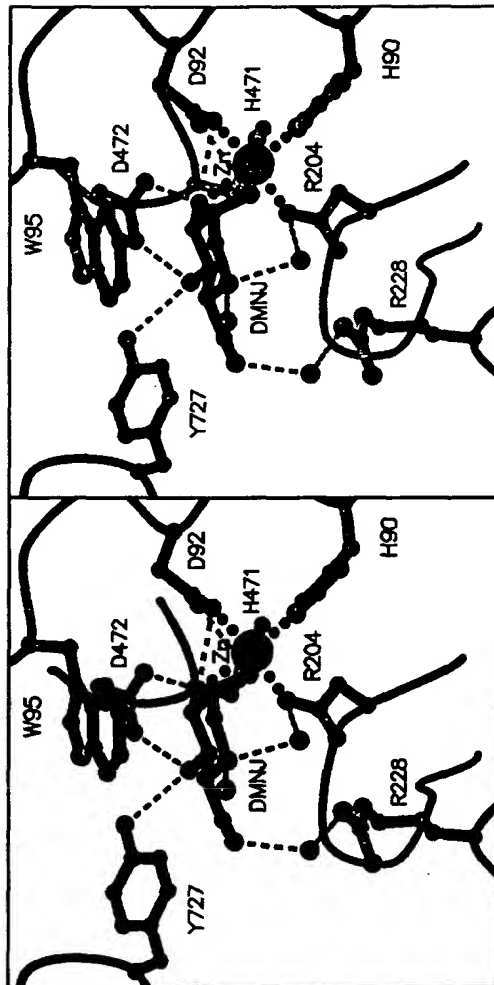


Figure 10B



Figure 10C

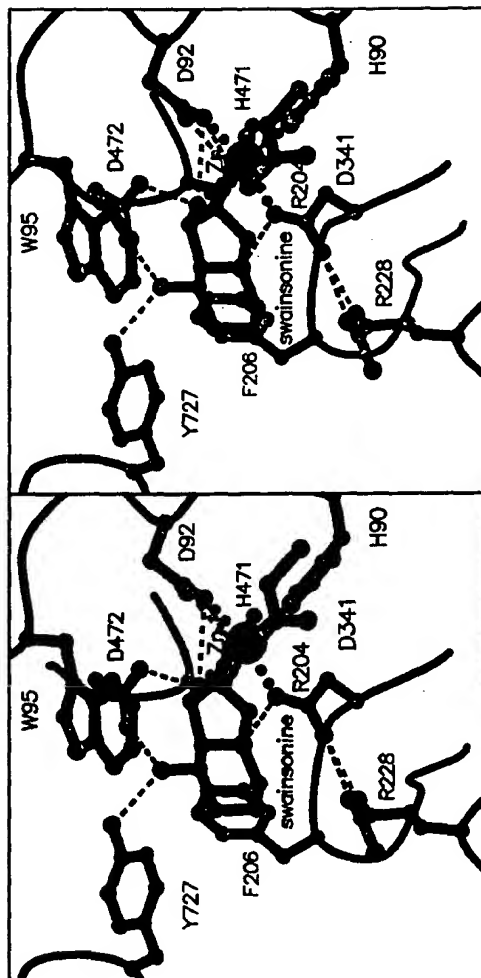


Figure 11A

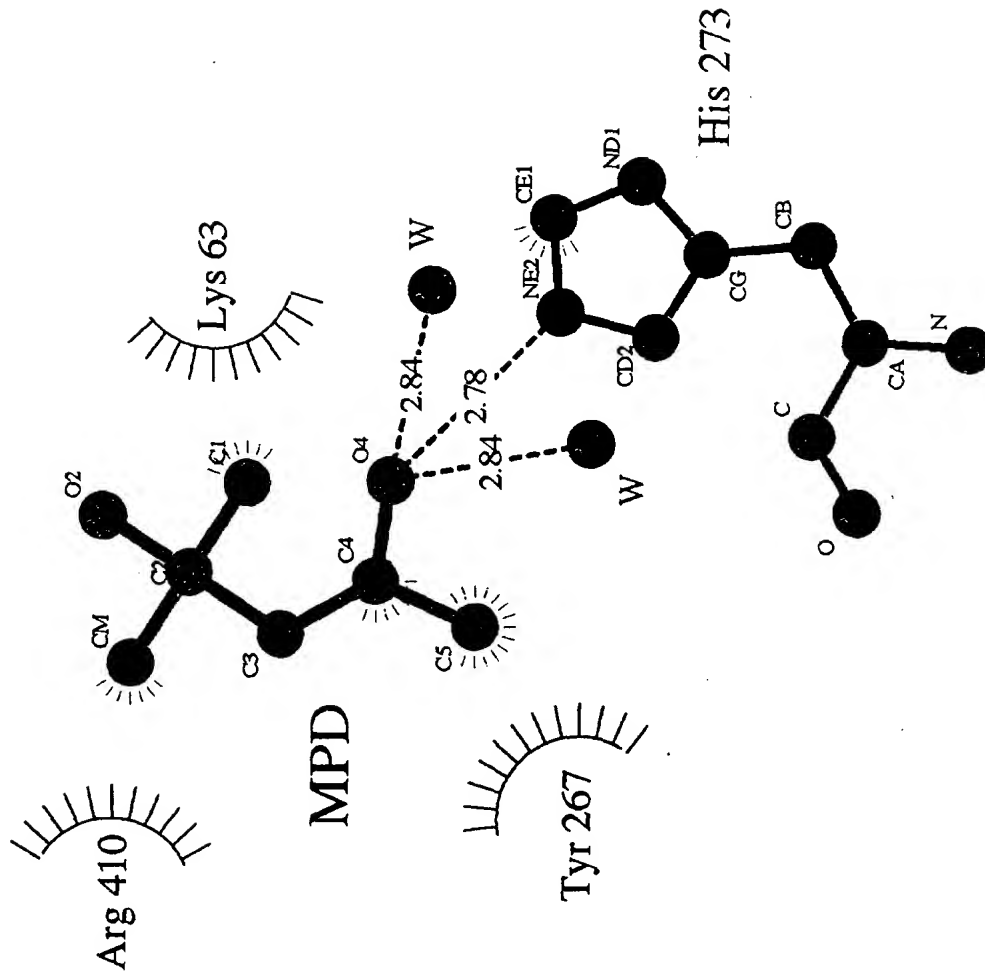




Fig. 11B

FIG. 11C

Figure 11C

